The effect of pre-soaking and time in the ultrasonic cleaner on the cleanliness of sterilized endodontic files

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Abstract

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Aims To assess whether pre-soaking files in an enzymatic cleaner prior to ultrasonic cleaning had any effect on cleanliness and also to assess the effect of the time that endodontic files spend in an ultrasonic bath prior to sterilization on their overall cleanliness.

Methodology Twenty root canals in a total of ten patients were cleaned and shaped using conventional techniques. Following use, some of the files were pre-soaked and then ultrasonically cleaned for either 5, 10, 30 or 60 min. Other files had no pre-soaking and were then ultrasonically cleaned. There were two control groups, one where the files were pre-soaked and not ultrasonically cleaned and the other where the files were neither pre-soaked nor ultrasonically cleaned. All files were then subjected to a standard packing and autoclaving process. Following autoclaving, the files were examined using a light microscope at a magnification of 40×. The cutting section of each file was divided into two parts, the tip and the shaft, for visualization under the microscope. Any debris or cement on the files was scored using a modification of the scale used by Smith *et al.* (*Journal of Hospital Infection*, **51**, 2002, 233). The data were analysed using one-way analysis of variance.

Results Pre-soaking had no significant effect on the cleanliness of the files ($P = 0.18$ at the tip, $P = 0.93$ at the shaft). Ultrasonic cleaning had a significant effect on the cleanliness of the files ($P < 0.00$) but there was not a linear relationship between cleanliness and the ultrasonic cleaning time. There was little benefit in extending the ultrasonic cleaning time beyond 5 min. Calcium hydroxide deposits on two files were resistant to ultrasonic cleaning.

Conclusions There is no benefit in pre-soaking endodontic files prior to ultrasonic cleaning. The optimum time for ultrasonic cleaning was between 5 and 10 min. Further ultrasonic exposure, up to 60 min, did not improve cleanliness. Although a majority of files were free from debris following ultrasonic cleaning, a substantial minority still retained debris. This supports the case for endodontic files being single-use only.

Keywords: endodontic instruments, endodontics, sterilization, ultrasonic cleaners.

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Introduction

The emergence of variant Creutzfeldt–Jacob disease (vCJD) has heightened awareness of the need for rigorous infection control precautions in all healthcare environments. From the healthcare perspective, vCJD is of concern because at present it is an incurable, fatal disease and the causative agent, an abnormal prion protein, is resistant to conventional inactivation procedures (Rutala & Weber 2001). vCJD infectivity in tissues encountered in dentistry, e.g. dental pulp, is implied by some animal models (Department of Health 2003). Though such infectivity has not so far been

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detected in humans, the possibility cannot be ruled out (Bebermeyer *et al.* 2003). Furthermore, there is evidence that some instruments used in endodontics, e.g. files and reamers, are particularly difficult to clean, and may carry significant material residues after washing (Gill *et al.* 2001). This might pose a threat of transmission risks if this residue were to carry vCJD infectivity (Department of Health 2003).

The safest and most unambiguous method of ensuring that there is no risk of residual infectivity on surgical instruments is to destroy them by incineration (Scully *et al.* 2003). However, traditionally instruments are sterilized and reused after treatment. A critical factor in deciding whether endodontic files should be single use or reusable is whether they can be satisfactorily cleaned prior to appropriate sterilization.

All instruments should be mechanically cleaned prior to sterilization to remove adherent materials on the external surface to minimize the risk of cross-infection. Ultrasonic cleaning is recommended because it reduces direct handling of decontaminated instruments, decreases the chance of puncture injuries and has superior cleaning ability compared with other cleaning techniques (Murgel *et al.* 1990, Palenik 1993, Cafruny *et al.* 1995). Pre-soaking instruments in an enzymatic cleaner prior to ultrasonic cleaning has been shown to increase the effectiveness of cleaning (Sanchez & Macdonald 1995). There have been various recommendations as to the time that dental instruments should remain in an ultrasonic bath for optimum cleaning (Miller & Hardwick 1988, Miller 1993, Palenik 1993) but no previous research on the effect of ultrasonic cleaning time on the cleanliness of endodontic files.

The aims of this study were:

1. To assess whether pre-soaking of the files in an enzymatic cleaner prior to ultrasonic cleaning had any effect on cleanliness.

2. To assess the effect of the time that endodontic files spend in an ultrasonic bath prior to sterilization on the overall cleanliness of the instruments.

Methodology

Teeth were selected which required root canal treatment for reasons of caries. Twenty root canals in a total of ten patients were treated by postgraduate endodontic students in the School of Dentistry at the University of Manchester, UK. Some of the teeth treated were multi-rooted teeth. One previously unused set of six K-Flex® stainless steel files (size 15–40) (QED, Peterborough, UK) was used for each root canal treated. A standard endodontic access cavity was prepared using a diamond bur in a high-speed handpiece. Conventional cleaning and shaping of the root canals was carried out using the crown-down technique. Files of size 15, 20, 25, 30, 35 & 40 were sequentially taken to the full working length with continuous recapitulation before proceeding to the next file size. During instrumentation, the canals were copiously irrigated with 3.5% sodium hypochlorite solution. All the used files were kept in a file holder (Dentsply, Weybridge, UK), which was specially designed to undergo sterilization procedures including ultrasonic cleaning and autoclave sterilization. These file holders were kept in airtight plastic containers until they were subjected to the cleaning procedures.

Sample groups

The files were divided into the following groups, with each group having twelve files (two sets of six files – Nos 15–40):

Group 1: pre-soaking + 5 min of ultrasonic cleaning.

Group 2: pre-soaking + 10 min of ultrasonic cleaning.

Group 3: pre-soaking + 30 min of ultrasonic cleaning.

Group 4: pre-soaking + 60 min of ultrasonic cleaning.

Group 5: no pre-soaking + 5 min of ultrasonic cleaning.

Group 6: no pre-soaking + 10 min of ultrasonic cleaning.

Group 7: no pre-soaking + 30 min of ultrasonic cleaning.

Group 8: no pre-soaking + 60 min of ultrasonic cleaning.

Group 9: control; no pre-soaking + no ultrasonic cleaning.

Group 10: control; pre-soaking + no ultrasonic cleaning.

Pre-soaking of the files

Following use, the files in groups 1–4 were immediately pre-soaked in an enzymatic cleanser (Zymex, Sultan Chemists, Englewood, NJ, USA) for 5 min, rinsed under tap water and then kept in an airtight container prior to ultrasonic cleaning the next day. The files in group 10 were also pre-soaked but then passed directly for autoclave sterilization.

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Ultrasonic cleaning of the files

Each set of files in groups 1–8 was placed in the ultrasonic cleaner for the appropriate time (5, 10, 30 or 60 min). The solution used in the ultrasonic cleaner was Ultraclean 3 (Oro-Clean Chemie AG, Fehraltorf, Switzerland). The files were then rinsed under running water for 10–15 s and then placed in airtight containers prior to their transportation to the Central Sterile Supply Department (CSSD) for processing and autoclaving the next day.

Autoclave sterilization

At the CSSD, the file stands were packed using porous autoclave paper that permits steam penetration to the instruments. This packing was done in a sterile environment and the files were then subjected to a standard autoclaving procedure (134 °C for 3 min at 1 atmospheric pressure).

Visualization of debris

The sterilized instruments were visualized for any debris, blood or contaminants using a compound microscope (Leica Microsystems, Milton Keynes, UK). The examination was carried out in a clean and dust free environment to try to prevent contamination from dust particles in the air. The debris was visualized at a magnification of 40×. Each file was rotated 360° before scoring. A computer was attached to the microscope in order to save the pictures in the system and also to attain reproducibility of pictures if needed. The whole length of the file was not visible under the microscope under 40× magnification. Therefore the cutting element of the file, which was 17 mm in length, was divided into two equal halves, the tip and the shaft. Each half was photographed and scored for debris separately.

Debris scoring

The scale used to measure the amount of debris on the surface of the file was a modification of the scale used by Smith *et al.* (2002). The previously used scale was modified because it was not sufficiently discriminatory. Using the Smith *et al.* (2002) scale, a file that had only one or two specks of dentine debris present scored the same as a file that had 25% coverage of debris.

A scale of 0 to ++++ was therefore used, where:

0 = No debris on the surface of the file.

+ = 0–5% of the file contaminated with visible debris.

++ = 6–15% of the file contaminated with visible debris.

+++ = 16–25% of the file contaminated with visible debris.

++++ = >25% of the file contaminated with visible debris.

The scoring was blinded by a colleague handing the files to the scorer (SAA) in a random manner without revealing the identity of the group to which each file belonged. The computer attached to the microscope was used to cross check the scores recorded by the microscope. A random sample of files was re-examined a second time to check intra-examiner reliability.

Statistical analysis

The debris data was analysed using one-way analysis of variance (SPSS Version 11.5, Chicago, IL, USA).

Results

The debris scores were normally distributed at both the tip and the shaft of the file. Kappa scores for the intra-examiner reliability of the debris scoring were between 0.75 and 0.90, indicating excellent agreement (Landis & Koch 1977).

Significance of pre-soaking phase

To discover if there was any significant difference in the debris scores between pre-soaking and no pre-soaking of the files before ultrasonic cleaning, an analysis of variance was performed using a univariate test for debris scores at the tip and the shaft of the files. The results showed that there was no statistically significant difference between the debris scores on files that had been pre-soaked or not pre-soaked before ultrasonic cleaning ($P = 0.18$ at the tip and 0.93 at the shaft).

As there was no significant difference with pre-soaking of the instruments before ultrasonic cleaning, the data for the pre-soaked and the non pre-soaked instruments were combined together for the purpose of further statistical tests. The new groups were recoded as below:

Group A: group 1 (pre-soak + ultrasonic cleaning for 5 min) and group 5 (no pre-soak + ultrasonic cleaning for 5 min), 24 files.

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Group B: group 2 (pre-soak + ultrasonic cleaning for 10 min) and group 6 (no pre-soak + ultrasonic cleaning for 10 min), 24 files.

Group C: group 3 (pre-soak + ultrasonic cleaning for 30 min) and group 7 (no pre-soak + ultrasonic cleaning for 30 min), 24 files.

Group D: group 4 (pre-soak + ultrasonic cleaning for 60 min) and group 8 (no pre-soak + ultrasonic cleaning for 60 min), 24 files.

Group E: group 9 (no pre-soak + no ultrasonic cleaning) and group 10 (pre-soak + no ultrasonic cleaning), 24 files.

Significance of ultrasonic cleaning

The debris scores for the files that had been cleaned ultrasonically (groups A–D) were compared with those files that had not been ultrasonically cleaned (group E). The results are shown in Table 1 and show a highly significant statistical difference ($P = 0.000$ at the tip and at the shaft) with the ultrasonically cleaned files having lower debris scores. These results demonstrate the benefit of using an ultrasonic cleaner to remove debris.

Debris scores at the tip of the file

The mean debris scores for the different groups at the tip of the files are shown in Fig. 1. The files were generally clean after 5 min or more of ultrasonic cleaning. However, there was not a linear relationship between the amount of debris and the time in the ultrasonic cleaner. After 5 min of ultrasonic cleaning, 13 of 24 files (54%) were scored as having no debris. The number of files with no debris rose to 17 of 24 (71%) after 10 and 30 min of ultrasonic cleaning but was only 13 of 24 (54%) after 60 min.

Table 1 Univariate analysis of variance for ultrasonic cleaning of the tip and shaft of the files

Ultrasonic cleaning	d.f.	Mean square	F	Significance
Tip	4	17.397	21.476	0.000
Shaft	4	12.319	10.895	0.000

Debris scores at the shaft of the file

The mean debris scores for the different groups at the shaft of the files are shown in Fig. 2. There was generally more debris at the shaft than at the tip. The number of files with no debris was only 3 of 24 (13%) after 5 min of ultrasonic cleaning, 9 of 24 (38%) after 10 min, 7 of 24 (29%) after 30 min and only 5 of 24 (21%) after 60 min.

Comparison of debris scores at the tip and the shaft

The mean and standard deviation of the debris scores at the tip and the shaft of the files were calculated (Table 2). The scores at the shaft were consistently higher than those at the tip and this difference was statistically significant (Pearsons correlation test $P < 0.000$).

Significance of different exposure time in ultrasonic cleaner

The data was subjected to one-way analysis of variance to compare the debris scores in the different groups for the different times in the ultrasonic cleaner (Table 3). The results showed a highly significant difference ($P < 0.000$).

For each group the mean debris score was calculated to detect the most effective method of cleaning. Using a

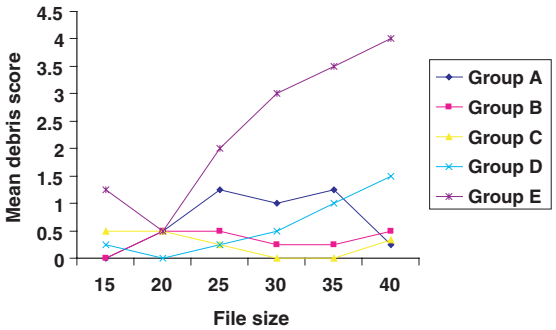

Figure 1 Debris scores at the tip of the file for groups A–E by file size.

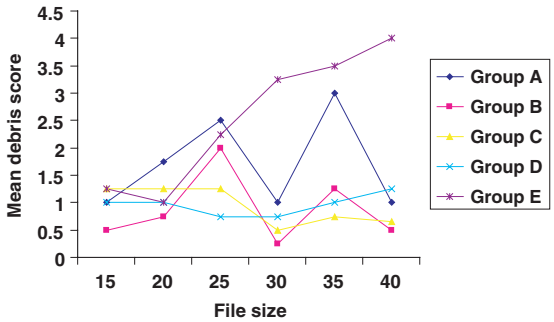

Figure 2 Debris scores at the shaft of the file for groups A–E by file size.

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Table 2 Means and Standard deviations (SD) of the debris scores in the different groups at the tip and the shaft of the files

Groups (ultrasonic cleaning time)	Tip	Shaft
5 min		
Mean	0.75	1.71
n	24	24
SD	0.94	1.23
10 min		
Mean	0.33	0.88
n	24	24
SD	0.57	0.9
30 min		
Mean	0.26	0.91
n	23	23
SD	0.45	0.95
60 min		
Mean	0.54	1.0
n	24	24
SD	0.78	0.83
No cleaning		
Mean	2.33	2.54
n	24	24
SD	1.40	1.25
Total		
Mean	0.85	1.41
n	119	119
SD	1.17	1.22

Table 3 One-way ANOVA table comparing different groups at the tip and the shaft

	Sum of squares	d.f.	Mean square	F	Significance
Tip					
Between groups	69.718	4	17.429	21.701	0.000
Within groups	91.560	114	0.803		
Total	161.277	118			
Shaft					
Between groups	49.456	4	12.384	11.243	0.000
Within groups	125.368	114	1.100		
Total	174.824	118			

post hoc Bonferroni multiple-comparison test applied to the ANOVA table, it was possible to identify which pairs of means differed. This is illustrated in Table 4 for the tip of the files and Table 5 for the shaft.

One-way analysis of variance was performed to compare the effect of the different ultrasonic cleaning times on the different file sizes but no significant difference was found ($P > 0.05$).

Calcium hydroxide debris

Two of the files (group A file size 25 and group D file size 40) had obviously been used by the operator to place calcium hydroxide paste up the root canal as an inter-appointment medicament. The paste was distinctly recognizable by its white colour and texture (Fig. 3). It was resistant to removal in the ultrasonic cleaner and therefore these files had high debris scores.

Discussion

An important cause of spread of infection from one person to another is use of contaminated instruments (Gurevich *et al.* 1996). The trend in health care settings is moving towards single use instruments. It has been suggested that endodontic files should be single use only, but because of cost implications this has not yet been implemented. As in the majority of dental practices endodontic files are considered as re-usable instruments, their cleaning and sterilization is of paramount importance. A recent study has shown that complete removal of organic debris from rotary nickel-titanium endodontic files is possible using a combination of cleaning procedures (moist storage, brushing followed by immersion in 1% sodium hypochlorite, ultrasonic cleaning) but this requires a meticulous technique (Linsuwanont *et al.* 2004).

This study has shown that the pre-soaking of files prior to ultrasonic cleaning does not produce any beneficial effect. This may be because most current ultrasonic cleaner solutions are prepared for use as both a pre-soaking solvent and a cleaning solution.

The cleanliness of the files was not directly correlated with the time spent in the ultrasonic cleaner. There was a significant difference within the first 5–10 min of ultrasonic cleaning but no further improvement up to 1 h. The current recommendations of the manufacturers of ultrasonic cleaners are that instruments should be immersed for between 5 and 10 min. The results of this study support that recommendation with the higher time limit of 10 min being preferable to give greater cleanliness at the shaft of the instruments. Because of the magnification used, it was not possible to visualize all the cutting element of the file in one view. Therefore, the tip and the shaft of the cutting element were visualized separately. This turned out to be fortuitous as the study demonstrated different levels of cleanliness at the tip and the shaft, with the tip being cleaner. The control files demonstrated that the tip and shaft had similar levels of debris, so the superior cleanliness of the tip may be associated with greater movement of the tip producing more ultrasonic cavitation effect.

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Table 4 *Post hoc* multiple-comparison test between different groups at the tip (statistically significant results in bold type)

	Mean difference	Standard error	Significance	95% confidence interval	
				Lower bound	Upper bound
5 min					
10 min	0.42	0.259	1.00	−0.32	1.16
30 min	0.49	0.262	0.64	−0.26	1.24
60 min	0.21	0.259	1.00	−0.53	0.95
No cleaner	**−1.58**	**0.259**	**0.00**	**−2.32**	**−0.84**
10 min					
5 min	−0.42	0.259	1.00	−1.16	0.32
30 min	0.07	0.262	1.00	−0.68	0.82
60 min	−0.21	0.259	1.00	−0.95	0.53
No cleaner	**−2.00**	**0.259**	**0.00**	**−2.74**	**−1.26**
30 min					
5 min	−0.49	0.262	0.64	−1.24	0.26
10 min	−0.07	0.262	1.00	−0.82	0.68
60 min	−0.28	0.262	1.00	−1.03	0.47
No cleaner	**−2.07**	**0.262**	**0.00**	**−2.82**	**−1.32**
60 min					
5 min	−0.21	0.259	1.00	−0.95	0.53
10 min	0.21	0.259	1.00	−0.53	0.95
30 min	0.28	0.262	1.00	−0.47	1.03
No cleaner	**−1.79**	**0.259**	**0.00**	**−2.53**	**−1.05**
No cleaner					
5 min	**1.58**	**0.259**	**0.00**	**0.84**	**2.32**
10 min	**2.00**	**0.259**	**0.00**	**1.26**	**2.74**
30 min	**2.07**	**0.262**	**0.00**	**1.32**	**2.82**
60 min	**1.79**	**0.259**	**0.00**	**1.05**	**2.53**

Table 5 *Post hoc* multiple comparison test between different groups at the shaft (statistically significant results in bold type)

	Mean difference	Standard error	Significance	95% confidence interval	
				Lower bound	Upper bound
5 min					
10 min	0.83	0.303	0.07	−0.03	1.70
30 min	0.80	0.306	0.11	−0.08	1.67
60 min	0.71	0.303	0.21	−0.16	1.57
No cleaner	−0.83	0.303	0.07	−1.70	0.03
10 min					
5 min	−0.83	0.303	0.07	−1.70	0.03
30 min	0.04	0.306	1.00	−0.91	0.84
60 min	−0.13	0.303	1.00	−0.99	0.74
No cleaner	**−1.67**	**0.303**	**0.00**	**−2.53**	**−0.80**
30 min					
5 min	−0.80	0.306	0.11	−1.67	0.08
10 min	0.04	0.306	1.00	−0.84	0.91
60 min	−0.09	0.306	1.00	−0.96	0.79
No cleaner	**−1.63**	**0.306**	**0.00**	**−2.50**	**−0.75**
60 min					
5 min	−0.71	0.303	0.21	−1.57	0.16
10 min	0.13	0.303	1.00	−0.74	0.99
30 min	0.09	0.306	1.00	−0.79	0.96
No cleaner	**−1.54**	**0.303**	**0.00**	**−2.41**	**−0.68**
No cleaner					
5 min	0.83	0.303	0.69	−0.03	1.70
10 min	**1.67**	**0.303**	**0.00**	**0.80**	**2.53**
30 min	**1.63**	**0.306**	**0.00**	**0.75**	**2.50**
60 min	**1.54**	**0.303**	**0.00**	**0.68**	**2.41**

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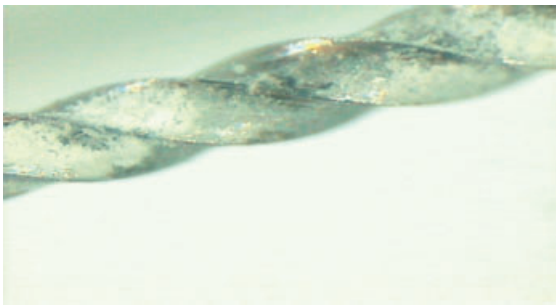

Figure 3 File with calcium hydroxide debris after 5 min of ultrasonic cleaning (40× magnification).

The cleaned instruments were sterilized in an autoclave with a standard sterilization procedure of 134 °C for 3 min. This has shown to kill most microbes, including spores, and is the most common method of instrument sterilization (Van Eldik *et al.* 2004). Prions would not be affected by this procedure but this study was designed to demonstrate the effectiveness of ultrasonic cleaners in cleaning files and not whether the files were sterile. A varying proportion of the files had residual debris after ultrasonic cleaning and this has potential implications for cross-infection and support the case for endodontic files being single-use only.

Two of the files had been used to transport calcium hydroxide into the root canal as an inter-appointment medicament. The ultrasonic cleaning did not appear to have any effect on this material and it was clearly visible after autoclaving. Further research is needed to clarify the most efficient method of removing this commonly used endodontic material from endodontic instruments.

Conclusions

No improvement in cleanliness was found with pre-soaking of endodontic files prior to ultrasonic cleaning and sterilization. The solutions presently recommended for use in ultrasonic cleaners appear to fulfil a dual role as an enzymatic cleaner and a detergent. The optimum ultrasonic cleaning time for endodontic files is between 5 and 10 min. Not all files were free from debris even with ultrasonic cleaning of up to 1 h and this finding supports the case for endodontic files being single-use only. Calcium hydroxide paste appears to be resistant to ultrasonic cleaning.

References

Bebermeyer RD, Powell JF, Hobdell MH, Durban EM (2003) Dental practice implications of prion diseases. *Quintessence International* **34**, 38–44.

Cafruny WA, Brunick A, Nelson DM, Nelson RF (1995) Effectiveness of ultrasonic cleaning of dental instruments. *American Journal of Dentistry* **8**, 152–6.

Department of Health (2003) *Transmissable Spongiform Encephalopathy Agents: Safe Working and the Prevention of Infection. Guidance from the Advisory Committee on Dangerous Pathogens and the Spongiform Encephalopathy Advisory Committee*. London: Department of Health. 15th December 2003.

Gill DS, Tredwin CJ, Gill SK, Ironside JW (2001) The transmissible spongiform encephalopathies (prion diseases): a review for dental surgeons. *International Dental Journal* **51**, 439–46.

Gurevich I, Dubin R, Cunha BA (1996) Dental instrument and device sterilization and disinfection practices. *Journal of Hospital infection* **32**, 295–304.

Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. *Biometrics* **33**, 159–74

Linsuwanont P, Parashos P, Messer HH (2004) Cleaning of rotary nickel-titanium endodontic instruments. *International Endodontic Journal* **37**, 19–28.

Miller CH (1993) Cleaning, sterilization and disinfection: Basics of microbial killing for infection control. *Journal of American Dental Association* **124**, 48–56.

Miller CH, Hardwick LM (1988) Ultrasonic cleaning of dental instruments in cassettes. *General Dentistry* **36**, 31–6.

Murgel CAF, Walton RE, Rittman B, Pecora JD (1990) A comparison of techniques for cleaning endodontic files after usage: A quantitative scanning electron microscopic study. *Journal of Endodontics* **15**, 214–7.

Palenik CJ (1993) Using ultrasonic cleaners to reduce exposure to blood borne disease. *Journal of Massachusetts Dental Society* **42**, 121–4.

Rutala WA, Weber DJ (2001) Creutzfeldt-Jakob disease: recommendations for disinfection and sterilization. *Clinical Infectious Diseases* **32**, 1348–56.

Sanchez E, Macdonald G (1995) Decontaminating dental instruments: testing the effectiveness of selected methods. *Journal of American Dental Association* **126**, 359–66.

Scully CJ, Smith AJ, Bagg J (2003) Prions and the human transmissible spongiform encephalopathies. *Dental Clinics of North America* **47**, 493–516.

Smith A, Dickson M, Aitken J, Bagg J (2002) Contaminated dental instruments. *Journal of Hospital Infection* **51**, 233–5.

Van Eldik DA, Zilm PS, Rogers Ah, Marin PD (2004) Microbiological evaluation of endodontic files after cleaning and steam sterilization procedures. *Australian Dental Journal* **49**, 122–7.

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